Data Evaluation Report on the toxicity of BAS 510 F to fish, early life cycle, Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number 2001-1027 **EPA MRID Number {454050-06}**

Data Requirement: PMRA DATA CODE: 9.5.3.1
EPA DP Barcode: D278418
OECD Data Point:
EPA Guideline: 72-4a

Test material: BAS 510 F **Purity (%):** 95.3%
Common name: Nicobifen
Chemical name
IUPAC: 2-chloro-N-(4'-chlororobiphenyl-2-yl) nicotinamide
CAS name: 3-Pyridinecarboxamide, 2-chloro-N_(4'-chloro[1.1'-biphenyl]-2-yl)
CAS No.: 188425-85-6
Synonyms:

Primary Reviewer: Peter Takacs and Peter Delorme **Date:** April 9/02
{PMRA}

Secondary Reviewer(s): Thomas M. Steeger, Ph.D. **Date:** July 20, 2002
{EPA} Thomas M Steeger

Company Code: BAZ
Active Code: CHH-BAZ-4
Use Site Category: In Canada, this fungicide is proposed for use on USC 13, 14 and 30; agricultural feed, food and turf uses. BAS 510 F is to be used 2-6 times per growing season depending on the crop, at a maximum recommended application rate of 875 g a.i./ha/application.

EPA PC Code: 128008

CITATION: Dr. S. Zok, December 20,1999. BAS 510 F - Early Life-Stage Toxicity Test on the Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) Department of Toxicology of BASF Aktiengesellschaft D-67056 Ludwigshafen/Rhein, FRG. Study number: 52FO179/975051

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EXECUTIVE SUMMARY:

The 97-day chronic toxicity of BAS 510 F to early life stage of rainbow rrout (*Oncorhynchus mykiss*) was studied under flow-through conditions. Fertilized eggs (25 x 4) of rainbow trout were exposed to control, and test chemical measured concentrations of 0.116, 0.241, 0.473, 0.891, and 1.667 mg a.i/L. The test system was maintained at 10 °C and a pH of 7.8 to 8.3. The 97-day NOEC and LOEC values, based on mortality and sublethal effects (narcosis and lethargy), were 0.116 and 0.241 mg a.i/L, respectively. Sublethal effects were not observed in the lowest treatment group (0.116 mg ai/L). In all other treatment groups (≥0.241 mg ai/L) lethargy and narcosis were apparent and were considered to be substance related. Also, one week after termination of hatch, extended yolk sacs were observed in the same treatment groups. The most sensitive sublethal endpoint was lethargy and narcosis (NOEC = 0.116 mg ai/L).

This toxicity study is classified as supplemental and does not fulfill guideline test requirements since there is uncertainty in whether the high water hardness and pH may have affected the toxicity and/or solubility of the test material. Additionally, EPA is uncertain regarding the actual exposure to dissolved phase BAS 510 F since water samples were not adequately processed prior to analysis. EPA however, is not requiring that the study be repeated at this time.

Results Synopsis

Test Organism Size/Age: fertilized eggs/embryos/larvae
Test Type: Flow-through
LOEC:0.241 mg a..i./L (without centrifugation); 0.25 mg/L nominal
NOEC: 0.116 mg a.i./L (without centrifugation); 0/125 mg/L nominal
Endpoint(s) Effected: 97-day survival, sublethal effects (narcosis, lethargy)

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was performed according to the OECD Guideline for Testing of Chemicals No. 210, adopted July 17, 1992 ("Fish Early-Life Stage Toxicity Test") and the (U.S.) EPA-FIFRA 72-4 (a), 1982 requirements considering the SEP (= Standard Evaluation Procedure) "Fish Early Life-Stage Test", by M. Rexrode and T.M. Armitage, U.S. EPA, Office of Pesticide Programs, Hazard Evaluation Division, EPA 540/9-86-138, July 1986.

COMPLIANCE:

This study was conducted in accordance with the GLP-provisions of the "Chemikaliengesetz" (Chemicals Act; Bundesgesetzblatt Jahrgang 1994, Teil I, 29.07.94; FR Germany) and with the "OECD Principles of Good Laboratory Practice" (Paris, 1981).

A. MATERIALS:

1. Test Material BAS 510 F

Description: Solid powder
Lot No./Batch No. : N 26
Purity: 95.3%
Stability of Compound: Stable in storage
Storage conditions of test chemicals: ambient conditions (room temperature)

Physicochemical properties of BAS 510 F.

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L*	low solubility
Vapour pressure	$7x10^{-9}$ mbar @ 20 °C	not volatile
UV absorption	UV molecular extinction: $1.53x10^3$ at 290 nm	-
pKa	does not dissociate in water	-
Kow	2.96	Not likely to bioconcentrate

*Study report gives solubility of BAS 510F in distilled water as 10.9 mg/L at 23°C. This value is roughly 2.7 times greater than previous studies have indicated . Even with a co-solvent the BAS 510F is sparingly soluble at 3 mg/L.

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Age /embryonic stage at test initiation:
The eggs were placed in the exposure chambers approximately 3 hours after fertilization and all embryos appeared to be in good condition at the beginning of the study.

(EPA requires fish embryos 2 to 24 hours old)

Method of collection of the fertilized eggs:
Eggs of 4 female and sperm of 4 male fish were used to produce the test specimens. After fertilization at the trout breeding farm on January 19, 1999, the eggs were transported to the testing facility.

Source:
Eggs and sperm of the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) were obtained from parent animals of the trout breeding farm Eußerthal Petrihof, Erber Söhne, 76857 Eußerthal/Pfalz, Germany.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding study:
A study was carried out at concentrations showing an effect between 1 and 2 mg ai/L based on acute rainbow trout study.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	parents not used	
Number of fertilized eggs/embryos in each treatment at test initiation	25 per replicate with 4 replicates per treatment; therefore, 100 embryos per treatment; loading rate at end of study = 0.35 g/L	acceptable *(EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure)*
Concentration of test material: Nominal: Measured:	0, 0.125, 0.25, 0.50, 1.0, 2.0 mg /L 0.116 mg/L = 92.8%, 0.241 mg/L = 96.4%; 0.473 mg/L = 94.6%; 0.891 mg/L = 89.1% and 1.667 mg/L = 83.4% of the nominal values.	acceptable *(EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *- One concentration must adversely affect a life stage and one concentration must not affect any life stage.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values)*
Solvent (type, percentage, if used)	not used	

Parameter	Details	Remarks *Criteria*
		(EPA requires that solvent should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol. *OECD requires that solvent must have no effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L)*
Number of replicates Control: Solvent control: Treatments:	 4 4 4 plus a viability control (14 day) (viability control contained twice the number of embryos, *i.e.*, 200)	acceptable *(EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used)*

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Parameter	Details	Remarks *Criteria*
Test condition: Type of dilution system for flow through method: Flow rate:	flow -through A vessel containing 1,000 L test water was connected via tubes with the filter and a pump; dilution water flowed over 30 g of test material until a saturated solution was obtained 2.5 L per hour. Therefore, with a 9-liter volume in the tank, complete water exchange would occur every 3.6 hours. At this exchange rate, approximately 6.7 complete tank exchanges would occur in a 24-hr period.. Flow rate for viability control is roughly double 13 exchanges per 24-hr period.	acceptable *(EPA requires: intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used. Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%)*
Aeration, if any	stock solution was aerated prior to distribution into exposure tanks.	acceptable *(EPA requires: dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated)*
Duration of the test	97 days	acceptable *(EPA requires 60 days post hatch)*
Embryo cups, if used Type/material: (glass/stainless steel) Size: Fill volume:	glass cylinders, 10 cm x 12 cm with 2x2 mm stainless steel mesh covering top.	acceptable *(EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen)*
Test vessel Type/material: (glass/stainless steel) Size: Fill volume:	stainless steel aquaria inner dimensions: 29 cm long, 21 cm wide, 22 cm high (13.4 L). 9 liters	acceptable *(EPA/OECD requires all glass or glass with stainless steel frame)*

Parameter	Details	Remarks *Criteria*
Source of dilution water	The dilution water was non-chlorinated drinking water obtained from the municipal water works of the city of Frankenthal, purified through a charcoal filter and aerated (fish laboratory quality).	dechlorinated tap water must be analyzed for residual chlorine and test chemical analyses of must be conducted. *(EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. OECD accepts any water in which the test species show control survival at least as good as presented in SEP)*
Water parameters: Hardness: pH: Dissolved oxygen: Temperature (s) (record all the temperatures used for different life stages): Photoperiod: Salinity (for marine or estuarine species): Other measurements: Interval of water quality measurements:	 230-240 mg/L $CaCO_3$ 7.8-8.3 90-100% saturation (9.7 - 11.3 mg/L) 10 °C Until swim-up the embryos and larvae were kept in the dark after swim-up: 150 Lux under the lid) at a light cycle of 16 hours light and 8 hours darkness Temperature measured hourly; pH and DO measured twice weekly; hardness measured weekly.	hardness of test water was > 5x that recommended by the EPA but acceptable according to OECD. Range of pH exceeded EPA recommended range of 7.2 - 7.6. *(EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week. Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature. OECD requires DO concentration between 60 - 90% saturation. Salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously)*

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Parameter	Details	Remarks *Criteria*
Post-hatch details: When the post-hatch period began: Number of hatched eggs (alevins)/ treatment released to the test chamber On what day, the alevins were released from the incubation cups to the test chamber	 day 35 all day 41	acceptable *(EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup)*
Post-hatch Feeding: Start date: Type/source of feed: Amount given: not stated Frequency of feeding: not stated	Beginning at swim-up the larvae were offered newly hatched brine shrimp larvae As soon as it could be observed that they started eating they additionally received commercial high protein trout starter "Kronen Fish Aminostart", After termination of swim-up the young fish received additionally „Trouvit" trout starter obtained from the trout farm.	acceptable
Recovery of chemical: Frequency of measurement: LOQ: LOD:	 weekly, from alternating aquaria, not reported not reported	acceptable
Positive control {if used, indicate the chemical and concentrations}	none	
Fertilization success study, if any Number of eggs used: On what day the eggs were removed to check the embryonic development:	none	
Other parameters, if any	-	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks / Criteria
Parameters measured including the sublethal effects/toxicity symptoms	embryo survival day 0 - 27 (beginning of hatch) - embryo/sac fry survival days 27 - 35 (termination of hatch) - hatch rate days 0 - 35 (termination of hatch), related to number of eggs initially exposed (= 100) -survival of larvae days 35 - 55 (termination of hatch until end of swim-up - survival of larvae days 0 - 55 (beginning until end of swim-up) - survival of young fish days 55 - 97 (termination of swim-up until end of study) - survival of individuals from days 0 - 97 (beginning to termination of the study), related to eggs initially exposed (= 100) Differences in survival for each time interval analyzed are counted from the observation time of the start day of the interval to the observation time at the last day of the interval. - body weights (wet weights) at the end of the study - body lengths (total length) at the end of the study - The time span from the initiation of the study to the onset of hatch and the time until termination of hatch were determined. - The time span from termination of hatch to termination of swim-up (100%) was defined. The occurrence of sublethal effects, if any, in sac-fry larvae and young fish, such as loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration or change of behavior, etc. were determined visually generally at least on workdays and recorded.	*(EPA minimally requires:* *- Number of embryos hatched; Time to hatch;* *- Mortality of embryos,larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	all observations were daily weight and length measured at test termination.	
Water quality was acceptable (Yes/No)	yes	
Were raw data included?	Yes	
Other observations, if any	-	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Table 3: Effect of BAS 510 F on egg hatching and survival at different life stage of rainbow trout (*Oncorhynchus mykiss*)

Treatment (mg a.i./L) [measured]	% embryo survival (day 0-27)	% embryo survival (day 27-35)	time to hatch (day)		Juvenile-survival on day 97
			start of hatch	end of hatch	% mortality
Control (dilution water only)	96%	96.9%	32-33	34-35	15
0.116	95%	97.9%	30-32	32-34	14.1
0.241	99%	91.9%	31-32	34	28.3 ★
0.473	99%	93.9%	29-31	32-34	81 ★
0.891	98%	68.4% ★	27-29	32-34	97 ★
1.66	96%	0 %★	27	28-33	100 ★
NOEC	0.116 mg ai/L				

★ Statistically different compared to the control.

End of hatch defined as the day at which the last larvae in a replicate of a treatment group has hatched before hatch was terminated, i.e., remaining eggs removed, on day 35. Hatch was terminated after hatch of ≥ 95% of the surviving individuals of a test group.

Table 4: Effect of BAS 510 F on growth of juvenile fish.

Treatment (mg a.i./L) [measured]	time to swim up		length (cm)	length (%control)	wet-weight (g)	weight (%control)
	start of swim-up	end of swim-up				
Control (dilution water only), if used	day 48 - 52	day 55	4.68	100	0.98	100
0.116	day 48 - 51	day 55	4.64	99.2	1.01	102.2
0.241	day 49 - 52	day 55	4.60	98.4	1.03	105
0.473	day 52	day 55	4.05★	86.5	0.82★	83.4
0.891	day 52	day 55	3.47★	74.1	0.47★	47.4
1.66	all fish dead	all fish dead	-	-	-	-
NOEC	0.241 mg ai/L					

★ Statistically significant compared to controls

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

At the end of the study vertebral deformations were seen in 2 fish of the control group and in no fish of the concentration groups.

No abnormalities were seen in the lowest concentration group (0.125 mg/L). In the higher test groups lethargy and narcotic state were observed in yolk sac larvae and were considered to be clear substance-related effects. Starting approximately one week after termination of hatch, extended yolk sacs were observed in the concentration groups 2 (0.25 mg/L), 3 (0.5 mg/L) and 4 (1.0 mg/L). In the highest concentration group (2.0 mg/L) extended yolk sacs were observed already directly after hatch.

Thus, the NOAEC for sublethal effects is 0.125 mg/L (nominal concentration) and 0.116 mg/L (based on the mean analytically determined concentration) and the LOAEC is 0.25 mg/L (nominal concentration) and 0.241 mg/L (based on the mean analytically determined concentration).

C. REPORTED STATISTICS:

For the body weight and the length of the fishes the statistical evaluation was carried out using DUNNETT's test for a simultaneous comparison of several dose groups with the control group. For the embryo, larvae and fish survival, a pair-wise comparison of each dose group with the control group was carried out via the log-rank test.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Method: One Way Analysis of Variance, with Student-Newman-Keuls Method.

Monday, April 15, 2002, 16:31:26
Data source: Data 1 in Notebook
Normality Test: Passed (P = 0.397)
Equal Variance Test: Passed (P = 0.946)

Group	Mean	Std Dev	SEM
day 97	21.250	2.062	1.031
Col 9	21.250	3.304	1.652
Col 10	17.750	3.403	1.702
Col 11	4.750	2.217	1.109
Col 12	0.750	1.500	0.750

Power of performed test with alpha = 0.050: 1.000

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor:

Comparison	Diff of Means		p	q	P<0.01
day 97 vs. Col 12	20.500	5	15.742	Yes	
day 97 vs. Col 11	16.500	4	12.670	Yes	
day 97 vs. Col 10	3.500	3	2.688	No	
day 97 vs. Col 9	0.000	2	0.000	No	
Col 9 vs. Col 12	20.500	4	15.742	Yes	
Col 9 vs. Col 11	16.500	3	12.670	Yes	
Col 9 vs. Col 10	3.500	2	2.688	No	
Col 10 vs. Col 12	17.000	3	13.054	Yes	
Col 10 vs. Col 11	13.000	2	9.983	Yes	
Col 11 vs. Col 12	4.000	2	3.072	No	

The re-analysis of the 97 day survival data using a one way ANOVA indicated that all but the two lowest test concentrations (0.125 and 0.25 mg ai/L) were statistically different compared to controls. However, the study authors determined that only the lowest concentration was statistically identical to the control. The authors used a log-rank test, (Kalbfleisch, J.D.; Prentice, R.L. (1980) which may be more sensitive than the method used here. The original results will therefore be used.

survival at test termination (97 day)
NOEC: 0.116 mg ai/L
LOEC: 0.241 mg ai/L

E. STUDY DEFICIENCIES:

The test water hardness was much higher (5.5x) than recommended by the EPA; dilution water pH exceeded EPA's recommended range. Dechlorinated tap water was used. Analytical measurements of treatment concentrations were not preceeded by centrifuging or filtering of the water samples. Level of quantitation (LOQ) and level of detection (LOD) not reported. Percent recovery of analytical standards from analysis method not reported.

F. REVIEWER'S COMMENTS: Similar to previous acute studies of BAS 510F by this testing facility, stock solution was prepared by circulating water over a filter containing 30 grams of test material until a saturated solution was obtained. In previous studies, saturated solutions were obtained at around 3.5 mg/L. This value was consistent with aquatic toxicity tests from other labs showing that BAS 510F is sparingly soluble at this concentration. In this study though, concentrations in the stock solution are reported to range from 3.11 to 6.13 mg/L and exceed what has previously been reported to be the solubility limit (4.69 mg/L). This study however reports that the solubility limit is 10.9 mg/L which is also inconsistent with other studies on the same compound. Given that the stock solution concentration varied by roughly a factor of 2 suggests that solubility of the test material is within the questionable range and is evidenced in the decline in percent recovery as concentrations of BAS 510 F increased. Chemical analysis should have been preceeded by centrifugation and/or filtration to assure that all of the test material was in the dissolved phase.

Water hardness (230 - 240 mg/L as $CaCO_3$) and pH (7.8 - 8.3) exceed EPA's recommended ranges of 40 - 48 mg/L and 7.2 -

7.6, respectively.

G. **CONCLUSIONS:** The study is classified as supplemental since it is unclear whether water hardness and pH affected the solubility and/or toxicity of BAS 510F. Also, it is uncertain whether measured concentrations of test material accurately reflect dissolved-phase concentrations since the water samples were not properly centrifuged and/or filtered prior to analysis.. The 97-day NOEC and LOEC for the most sensitive endpoints, survival and sublethal effects (narcosis, extended yolk sacks, and lethargy) were 0.116 and 0.241 mg ai/L (mean "measured" concentration), respectively. EFED is not requiring that this study be repeated at this time.

III. REFERENCES:

Kalbfleisch, J.D.; Prentice, R.L. (1980): The statistical analysis of failure time data. John Wiley & Sons, New York.

Approved 04/01/01 C.K.